Hello, this is Kevin O'Donovan, and I'd like to welcome you to the National Institute of Environmental Health Sciences Superfund Research Program monthly Research Brief podcast.

This month we're heading to sunny San Diego, where Dr. Julian Schroeder has discovered the key to metal accumulation in plants.

The research brief, number 192, was released on December 1, 2010, and was written by SRP contractor Maureen Avakian in conjunction with SRP-supported researcher Dr. Julian Schroeder from the University of California – San Diego.

Heavy metals such as mercury, lead and cadmium and the metalloid arsenic are among the 10 most hazardous substances found at U.S. Superfund sites. To survive in environments with elevated levels of toxic heavy metals or arsenic, plants have developed mechanisms to sense and detoxify these non-essential substances. In a 1985 publication in *Science*, researchers reported the discovery of phytochelatins, small peptides that are synthesized when toxic heavy metals or arsenic enter plant cells. Phytochelatins bind metals in the cytosol and the heavy metal-phytochelatin complexes are actively transported into the vacuoles. The metals are concentrated and sequestered in vacuoles, protecting the other cellular structures. However, this mechanism also results in the potential for human intake and exposures – heavy metals often enter the human food chain when people consume crop plants grown in heavy metal contaminated soil or irrigated with contaminated water.

In the 25 years since the discovery of phytochelatins, researchers around the world have worked to identify the vacuolar phytochelatin-heavy metal transporter genes. This knowledge could serve as the foundation to develop strategies to alter tissue-specific expression of phytochelatin transporters to (1) improve crops to prevent/minimize the uptake of toxic heavy metals, or (2) engineer non-crop plants capable of removing toxic metals from soil and water at hazardous waste sites (phytoremediation / phytoextraction). One of the main goals of Dr. Julian Schroeder's Superfund Research Program-supported research at the University of California – San Diego is to characterize transporters of toxic metals. Several years ago, Schroeder's laboratory, in parallel with two other labs, discovered the gene for the key enzyme that synthesizes the heavy metal and arsenite detoxifying phytochelatins. However, identification of the essential vacuolar phytochelatin transporter genes remained elusive.

One phytochelatin transporter, Hmt1, has been identified in *Schizosaccharomyces pombe* (the "fission yeast"), but two lines of evidence suggested that others must exist. First, Hmt1 knockout strains of *S. pombe* can accumulate significant phytochelatin levels in vacuoles. Second, whole-genome sequencing revealed that plant genomes lack Hmt1-like transporters yet plants have the ability to sequester heavy metal-phytochelatin complexes in vacuoles. Dr. David Mendoza-Cózatl, a senior postdoctoral scientist in Dr. Schroeder's laboratory, approached the challenge to characterize phytochelatin transporters logically – by using *S. pombe* as the focus of the search. This yeast strain has a much smaller genome than plants. It has only 11 ABC transporter genes

and only four (Hmt1, Abc2, Abc3, and Abc4) are targeted to the vacuolar membrane. A plant genome typically contains 120 or more candidate ABC transporter genes. The Schroeder research group, together with Paul Russell's UCSD Superfund Research group, conducted a systematic analysis of *S. pombe* deletion mutants, cadmium tolerance assays, analysis of phytochelatin content in purified vacuoles, and complementation experiments. They demonstrated that Abc2 and its encoded transporter are the long-sought vacuolar phytochelatin transporters, essential for cadmium tolerance in yeast and for cadmium-phytochelatin uptake into the vacuoles. They also found that Abc2 and Hmt1 have both distinct and overlapping functions in metal and xenobiotic detoxification.

In a separate study, Drs. Mendoza-Cózatl and Schroeder collaborated with researchers at the University of Zurich and POSTECH in South Korea to identify heavy metal-phytochelatin transporters in the plant *Arabidopsis thaliana*. This work was specifically focused on identifying mechanisms for arsenic accumulation and detoxification in plants with the ultimate goal of reducing nutritional arsenic intake through the consumption of contaminated plants. They demonstrated that two genes are vacuolar phytochelatin transporters, and are required for arsenic resistance in *A. thaliana*. When both plant genes were deleted from the plant genome, the plants could not accumulate arsenic in their vacuoles. These ABC transporters are plant orthologues of the Abc2 transporter identified in *S. pombe*.

These findings provide a key to understanding the detoxification of heavy metals and metalloids, in particular arsenite, that are conjugated with phytochelatins for detoxification. The research provides long-sought after identification of major vacuolar phytochelatin transporters. Modulation of these transporters may allow researchers to engineer plants suited either for phytoremediation or reduced accumulation of arsenic in edible plant materials, such as grains and fruits.

Dr. Schroeder believes that among other applications, "Future work may enable scientists to use the technology to avoid arsenic accumulation in rice grains, which exposes millions of people to arsenic, increasing incidence of cancer in large populations in India, Bangladesh and other countries."

If you'd like to learn more about this research, visit the Superfund Research Program website at www.niehs.nih.gov/srp. From there, click on "Who We Fund" and follow the links to the University of California – San Diego's research summary. If you have any questions or comments about this month's podcast, or if you have ideas for future podcasts, contact Maureen Avakian at avakian@niehs.nih.gov.

Join us next month as we discuss amending current Superfund site cleanup methods to accelerate the removal of contaminants.